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Editorial

Pragmatics and Statistics in Psychiatric Genetics

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This journal recently published a multicenter study (which I coordinated) of a schizophrenia sample from fourteen sites, in which modestly positive lod scores were interpreted (by consensus) as somewhat supportive of linkage on chromosomes 6p and 8p (SLCG, 1996). The next issue included three commentaries (Kidd; 1997, Crow, 1997; Rice, 1997) dealing in part with issues related to statistical inference. I will argue here that in the context of an evolving pragmatic approach in this field, it will be useful to consider genome-wide levels of significance as advocated by Lander and Kruglyak (1995), but that caution is required in relying on predetermined thresholds of significance rather than on consideration of methods and patterns of results within and across studies.

REAL PROGRESS DESPITE OBSTACLES

Crow lists twelve examples of positive schizophrenia linkage reports, none statistically compelling and each contradicted by others. He criticizes a premature acceptance of the hypothesis of many susceptibility loci of small effect, which he believes inhibits the questioning of contradictory reports. Informal discussions suggest, to the contrary, that colleagues understand that many findings will prove to be false positives, and believe that loci with some substantial effect on susceptibility will be found. There is both concern and disagreement about how best to use statistical evidence in the search for true positives.

Schizophrenia linkage studies face obstacles (noted by Kidd and by Rice) that include imprecise diagnostic methods, a variable phenotype, unknown mode of transmission, lack of familial subtypes, and potential errors in specifying marker allele frequencies. Nevertheless, a set of studies, most initiated around 1990 using diverse methods, have made slow but substantial progress. Media reports notwithstanding, many of us expected real progress to take a decade. After seven years we have many samples, better maps, genotyping and statistical methods, and several positive findings which have received support from independent samples, including 6p (Straub et al., 1995; supported by Schwab et al., 1995; Moises et al., 1995; SLCG, 1996 [which partially overlaps with Moises's and includes Schwab's sample]), 8p (Pulver et al, 1995; then Kendler et al., 1996; SLCG, 1996 [includes the Kendler sample]), 22q (Pulver et al.; then Gill et al., 1996), 13q (Lin et al., 1995; then Antonarakis et al, 1996) and 9q and 20p (initial and follow-up findings in Moises et al., 1995). There is also a linkage for a putative trait marker, deficient pre-pulse inhibition of the P50 EEG potential, (Freedman et al., 1997). Some or all of these findings may be false positives, but some (or others still to come) may prove real.

A PRAGMATIC APPROACH

Initial skepticism about schizophrenia genome scans (based on epidemiological data favoring polygenic inheritance for which linkage methods are not ideal), was counterbalanced by a view that locus heterogeneity (a major locus in a proportion of families) could not be excluded without observing the results of scans. Scans completed to date (Coon et al., 1994; Moises et al., 1995; Levinson et al., 1996) and many partial reports do not reveal evidence for a single major locus. However, in light of initial promising evidence as discussed above, a pragmatic approach to the cloning of susceptibility genes has evolved, including evaluating positive linkage reports in additional samples, multicenter analyses, search for candidate genes in implicated regions, linkage disequilibrium mapping, mutation analysis and sequencing. There is a recognition that studies differ in terms of population, sampling strategy, explicit and implicit diagnostic model and analytic approach. Investigators prioritize their efforts somewhat subjectively based on their own and others' data. R. Straub points out (personal

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communication) that in the absence of striking linkages, each study still provides important rank-ordered information for follow-up studies. The magnitude of linkage scores, number of positive reports, and judgements about the size and methods of studies also influence priorities.

Time will tell whether linkage studies will lead to cloning of susceptibility loci without first demonstrating "significant" linkage; and whether weak linkages reflect weak effects on susceptibility or limitations of our methods to reveal the true magnitude of effects. We may find that more significant results are obtained with narrowing of chromosomal regions or clinical subgroups.

STATISTICAL INFERENCE

What approach to statistical inference will be most useful in this process? Inferences about monogenic traits are based on calculation of prior and posterior probabilities of linkage, with a lod score of 3 (~ 3.3 for linkage with heterogeneity) representing 20:1 posterior odds for linkage. But for complex disorders, prior probability of linkage is not known, and a different approach is needed. There have been proposals for corrected lod score thresholds, simulation strategies, and use of model-free tests which calculate deviation of the data from random sharing of marker alleles. A p value for a complex disorder should reflect the probability of the data in the absence of linkage. But there is little consensus about how to interpret "somewhat positive" statistical evidence from a genome scan, or a pattern of results from conflicting studies.

A current debate highlights some major issues. Lander and Kruglyak (1995) presented a formula for $\mu(T)$, the genome-wide level of significance or probability of observing a given point-wise p value by chance in a genome scan, based on the distribution of recombinations. Full inheritance information and a single multipoint test are assumed. They propose thresholds for "suggestive" (expected once per genome scan), "significant" (once in 20 scans) and "highly significant" (once in 1000 scans) linkage, arguing that "scientific disciplines erode their credibility when a substantial proportion of claims cannot be replicated". They suggest that new disease loci be named only when findings reach genome-wide significance, argue for a category of "suggestive linkage" because "adopting too high a hurdle for reporting results runs the risk that [a] nascent field will be stillborn", and suggest reporting of all regions with nominal $p < .05$ in a genome scan. The proposal has received considerable support.

Two aspects of these "guidelines" have been criticized (indeed some of the exchanges have been unusually, perhaps unnecessarily sharp in tone):

1. It has been argued that reporting of nominal p values, and informed consideration of their interpretation by authors and editors, are more useful than specific thresholds (Witte et al., 1996; Curtis, 1996; Elston, 1997).

2. It has been questioned whether it is valid to consider genome-wide significance when fewer markers have been analyzed (Witte et al., 1996; Elston, 1997), or when multiple linkage tests have been carried out (Curtis, 1996).

P VALUES AND THRESHOLDS

Recent experience supports the assumption that schizophrenia linkage analyses are undertaken within genome scans or to extend their findings, so that it seems useful to estimate, report and discuss one's interpretation of both nominal and genome-wide p values, based on the $\mu(T)$ formula or refinements to it, or on simulations. Improved programs for simulating multipoint data and analyzing them by model-free methods would facilitate work on this problem. The proposed threshold for statistically significant linkage -- a result expected to be observed by chance not more than once in twenty genome scans -- seems a reasonable one. But editorial policies should remain flexible (Curtis, 1996). It is most important that we as authors give enough information about the nature and number of linkage tests that produced a given point-wise or genome-wide result, to permit an informed interpretation by the reader. Elston (1997) makes a similar point, but I favor evaluation of complex disorder data in the context of genome-wide significance, while he does not.

The $\mu(T)$ formula cannot be applied directly to single-point or multiple tests, anticonservative statistics or small samples (all of which make results less significant than the formula predicts). If multiple tests are used, the effect on genome-wide significance deserves discussion. (The trend is toward use of one or a few model-free analyses.) It may be valuable (particularly in larger datasets) to test multiple diagnostic definitions; but weighting the contribution of diagnostic classes in a single test can sometimes preserve power (Terwilliger and Ott, 1994; Levinson, in press). Note that analyses of epistasis require careful thinking about false positives: a two-locus analysis based on a genome scan is the result of perhaps 300 x 300 or more tests, whether carried out formally or by "eyeballing" positive regions.

LOOKING ACROSS STUDIES

Unfortunately, in the absence of a significant linkage from a single or combined dataset, current statistical procedures can offer little guidance in interpreting mixtures of positive and negative findings. A threshold for "suggestive" linkage seems less useful than for significant linkage, because the pattern of results across studies becomes more critical: one might be more impressed with three findings of p = .004 in large samples, than with one finding of p = .001 in a very small sample. Consideration of genome-wide significance levels can remind us that our "somewhat positive" results can easily occur by chance (even more than once), but interpretation of such results always represents a gamble in one direction or the other. For example, Kidd remarks that the SLCG findings on 6p and 8p are too weak to warrant gene-cloning efforts, yet such efforts are underway in both regions. Again, time will tell.

Formal methods for evaluating multicenter data deserve further discussion. Lander and Kruglyak (1995) advocate pooling and re-analysis of datasets with the same markers and method, as in the SLCG (1996) and Gill et al. (1996) studies. Rice questions the validity of pooling data collected with different methods, and makes the valuable suggestion of using meta-analysis methods which parameterize these differences, but further work would be required to arrive at measures of relevant variations such as recruitment and diagnostic procedures or genotyping accuracy.

CONCLUSION

Both the pragmatic, rank-order-and-replicate approach and careful statistical inferences are needed in our endeavor. Will exact p values greatly influence our search for schizophrenia susceptibility genes? Possibly not, if genes are rapidly cloned in the regions with the most positive current findings. It is more likely that even if some such efforts succeed, many problems will remain. At that point we would be far ahead of 1990, but with many conflicting results and difficult decisions about where to invest resources. We will need continued progress in making statistical inferences about linkage (and linkage disequilibrium) data to make difficult choices and to preserve the credibility of the field while it struggles to reach its initial goals.

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